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***Full Length Article***

# Seed Quality of Two *Baccaurea* Species as Affected by Mucilagenous Seed Coat and Desiccation

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## Abstract

The present study evaluated seed quality of *Baccaurea polyneura* and *Baccaurea motleyana* as affected by mucilaginous seed coat (MSC) and desiccation, in order to obtain seed processing protocols and their storage behavior. Initially, seed quality with intact and removed MSC was compared. The seeds were then desiccated from initial moisture content (MC) to targeted MC of 40, 30, 20 and 10%. Data on viability, vigour and seedlings categorization for evaluation of normal and abnormal seedlings were recorded at 30 days of germination. Removing MSC increased germination by 12.62% in both species and improved mean germination time (MGT) and germination index (Gi) in *B. motleyana* only. Desiccation from 30% to 20% MC resulted no decrease in the germination of *B. polyneura* but reduced in *B. motleyana*. Reduction seedling categorized C and D (as normal seedlings) as MC reduced, was higher in *B. motleyana*. Higher correlation coefficient value ($r = -0.87$, $p \leq 0.01$) between MC and MGT in *B. motleyana* was found as compared to *B. polyneura* which indicated sensitivity of *B. motleyana* to desiccation. Removing MSC significantly enhanced seed viability of both species, but for vigor, was more profound in *B. motleyana*. Seeds of *B. motleyana* took longer time to desiccate and were more sensitive to desiccation, indicated by the seeds deterioration; viability and vigor. These preliminary information would suggest in terms of seed storage behavior, *B. motleyana* as recalcitrant while *B. polyneura* classified as intermediate. © 2020 Friends Science Publishers

**Keywords**: Baccaurea; Desiccation sensitivity; Seed quality; Seed storage; Underutilized fruits

## Introduction

Malaysia is one of richest countries in the world in terms of biodiversity per unit area, although it only covers 0.2% of the world's land mass. The 2001 Global Diversity Outlook recognized Malaysia as one of the 12 mega-diversity countries in the world (Mukrimah *et al.*, 2015). This priceless treasure however is being threatened due to deforestation, rapid agricultural development and industrialization accompanied by other social and cultural pressures. Malaysia is located at the Southeast Asia region. Albeit this region is highest ranked in the world biodiversity, it has the highest rate of deforestation compared to any other tropical region where approximately 75% of the original forest and 42% of its biodiversity is expected to be lost by 2100 (Sodhi *et al.*, 2010). According to a new global forest map, Malaysia had the world's highest rate of forest loss between 2000 and 2012 (Salma *et al.*, 2015). This scenario has built an alarm for conservation programs to be taken seriously by government and community.

In conserving plant genetic material, particularly seed, it is important to know whether the species shows orthodox, intermediate or recalcitrant seed storage behavior. Orthodox seeds face dehydration at the point of maturation and can be preserved at low moisture content and low temperature for extended lengths of time. These seeds are often found in the temperate quarters of our planet (Roberts, 1973). Recalcitrant seeds show no occurrence of maturation-related drying and the seeds are shed off at moderately high moisture content (Chin, 1988). They are incapable to withstand extensive desiccation. Since they exhibit vulnerability to chilling, storing them using conventional seed storage methods is almost impossible. The term intermediate seed on the other hand was coined by Ellis *et al.* (1990) for seeds that did not account satisfactory for all observations on seed storage behavior; orthodox or recalcitrant. Recalcitrant and intermediate seeds usually originate in the tropics such as oil palm, coffee, cacao, rubber and some tropical fruits. To maintain the viability of this type of seeds, the factors that determine the level of dehydration sensitivity have to be considered and the seeds preserved in a moist and moderately warm environment; nonetheless, even under such favourable conditions, the lifespan of these seeds is restricted to days and infrequently months.

*Baccaurea polyneura* and *Baccaurea motleyana* or their respective common name jentik-jentik and rambai which belong to the Phyllantaceae family are few of the indigenous crops in Malaysia. These species can be easily found in Kedah, Terengganu, Kelantan and Negeri Sembilan. Throughout the world, there are 42 species of

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*Baccaurea* with 31 species can be found in Malaysia (Khadijah *et al.*, 2014). The edible fruit from *Baccaurea* species serve as source of additional nutrients and reported to be high in antioxidant activities (Nurhazni *et al.*, 2013; Khadijah *et al.*, 2018). Sustainable utilization of *Baccaurea* species in the supply chain of supplement and medicine is highly dependent on the successful propagation of this tree species. Currently, no other means of propagation is feasible for these species except via seed. Since *Baccaurea* species produce fruit seasonally, there is a need to store the seeds to ensure production which can be carried out all-year round. However, information on storage methods of their seed is still lacking. Previous study by Normah *et al.* (1997) had reported seed viability of *B. polyneura* and *B. motleyana* seeds when subjected to desiccation. Nevertheless, effect of mucilaginous seed coat (MSC) on seed quality was not studied and no information on seedling vigor was reported in the study. Data on seedling vigor would serve a better prospect in terms of classifying seed storage behavior of *B. polyneura* and *B. motleyana.* Therefore, this study was conducted to evaluate see6d quality; viability and vigor of *B. polyneura* and *B. motleyana* as affected by desiccation, in order to obtain preliminary information on their storage behavior. This protocol will facilitate for effective seed processing technique prior to seed drying and storage.

## Materials and Methods

### Plant Materials

Fresh ripe fruits of *B. polyneura* and *B. motleyana* were collected around Kuala Nerang, Kedah on August 2018. The pericarp was removed from the fruits manually by washing under running water. For effect of MSC study, seed lots with intact MSC (control) and removed MSC for both species were prepared. The MSC was removed by soaking the seeds overnight in water before being scrubbed with metal mesh sponge. For desiccation study, seeds with removed MSC were used.

### Measurement of Moisture Content

Moisture contents (MC) of both species before and after desiccation were determined using the low constant temperature oven method and percentage of MC was calculated on a fresh weight basis as prescribed by International Seed Testing Association (ISTA, 2006). Four replicates of 30 and 20 seeds were used for *B. polyneura* and *B. motleyana* respectively, in determining the MC. The aluminium containers were weighed ($W_1$) and the depericarped seeds were then cut into small pieces and placed into the container. The weight of the fresh seeds and the container were then weighed, recorded ($W_2$) and placed in the oven for 17±1 h with temperature of 103±1°C. The weight of dried seeds and the containers were measured and recorded ($W_3$). The MC was measured based on the weight of water loss during drying in oven and calculated based on formula below:

$$\% \text{ Moisture content (MC)} = (W_2 - W_3) / (W_2 - W_1) \times 100$$

$W_1$ = Weight of aluminium container (g)
$W_2$ = Weight of aluminium container + fresh seeds (g)
$W_3$ = weight of aluminium container + dried seeds (g)

### Desiccation Treatment

Seed of *B. polyneura* and *B. motleyana* with removed mucilaginous seed coat were placed on a modified culture rack for even desiccation in the laminar air flow. The sample weight were monitored periodically until the seeds MC reached 40, 30, 20 and 10%. When the sample weight had reached the targeted weight, the seed lots were sealed in hermetic storage and the actual MC of the seeds were determined using low constant temperature oven method. Desiccation period, hour (h) for the seeds to reach targeted MC was recorded and the desiccation rate was calculated and compared between the species. The weight of sample seeds for targeted MC was calculated as below:

$$\text{Target weight, } W_2 \text{ (g)} = W_1 - [W_1 \times (MC_1 - MC_2) / (100 - MC_2)]$$

$W_1$ = Initial weight (g)
$MC_1$ = Initial MC (%)
$MC_2$ = Target MC (%)

### Seed Germination and Vigour Test

The sterilized sand was moistened using distilled water to 50% of the sand water holding capacity and placed in plastic boxes. The seeds were then treated with powdery Benlate accordingly. Four replicates of 20 seeds for *B. motleyana* and 4 replicates of 30 seeds for *B. polyneura* were spaced out in the media and covered with 10 – 20 mm of loose sand. The seeds were kept in germination chamber (25 ± 2°C, 75±2% RH) under 12-h light/12-h dark with light photon flux density of 26-30 $\mu$moL m $^{-2}$ s $^{-1}$ and watered daily using distilled water. The numbers of germinated seeds for both species were recorded at 30 days of germination. The seeds were considered as germinated when the seedlings emerge from sand surface. Percentage of germination (GP%), mean germination time (MGT) and germination index (Gi) were calculated as below:

G% = Total number of germinated seeds x 100

MGT = $\Sigma$ Fx / $\Sigma$ F, where F is number of seed germinated on day x
Gi = $N_1 / D_1 + \ldots + N_L / D_L$, $N_1$: Number of seeds germinated on 1st count, $D_1$: Days to 1st count, $N_L$: Number of seeds germinated on last count, $D_L$: Days to last count

### Morphological Grouping of Seedlings

For germination percentage, the seedlings for both species were grouped based on their morphological difference and were ranked into four categories i.e., A) un-germinated seeds; B) abnormal seedling; C) seedling with plumule still intact with testa and D) seedling with fully expended plumule (Fig. 1).

### Experimental Design and Data Analysis

For the MSC study, the treatments comprising of 2 species and 2 MSC condition were arranged in a CRD with four replications; 30 and 20 seeds per replication for *B. polyneura* and *B. motleyana* respectively. In desiccation study, the treatments comprising 2 species and 5 desiccation levels were arranged in a CRD with four replications; 2200 seeds per replication for both species. The data obtained was analyzed using ANOVA in the SAS software (Version 9.4, SAS Institute Inc. Cary, North Carolina, USA) and differences between treatment means were compared using Tukey's Honest Significant Difference (HSD) at P ≤ 0.05%. Pearson correlation coefficient (r) was determined between the variables in each species at P ≤ 0.05%.

## Results

### Mucilagenous Seed Coat (MSC) Effects on Seed Quality

Species had significant effect (P<0.05) for MGT and Gi while MSC had significant effect (P<0.05) on germination percentage and MGT. Significant interactions (P<0.05) between the main effects were recorded in all seed quality traits except for germination percentage (Table 1). Significant interactions between the main effects recorded in MGT and Gi revealed that effects of removing MSC on MGT and Gi were highly dependable on the species. Removing MSC did not significantly increase MGT and Gi in *B. polyneura*. However, in *B. motleyana*, seeds with removed MSC germinated significantly faster by 43.90% compared to seeds with intact MSC (Fig. 2A). The same pattern can be observed in Gi where *B. motleyana* seeds with removed MSC had significantly higher Gi by 114.9% as compared to seeds with intact MSC, whereas no significant difference was observed in *B. polyneura* (Fig. 2B).

### Desiccation Rate for *B. polyneura* and *B. motleyana*

When desiccated over time, both species followed exponential decay pattern where increase in desiccation time resulted in decrease of MC exponentially. The overall rate of desiccation however was different between the species. Desiccation rate in *B. polyneura* was 3.11 times faster as compared to *B. motleyana*. Seeds of *B. motleyana* took 52 h to be desiccated from initial MC, 54% to target MC of 20% whereas in *B. polyneura*, the seeds only took less than 15 h (Fig. 3).

### Effects of Desiccation on Seed Quality

Species and the presence/absence of the MSC significantly (P<0.05) affected germination percentage, MGT and Gi. Significant interactions (P<0.05) between species and MSC were recorded in GP and MGT, but not in Gi. Regardless of the species, desiccating the seeds from initial MC of 49.42% (*B. polyneura*) and 54.07% (*B. motleyana*) to 40% MC significantly reduced the Gi by 27.6%.

**Table 1:** Main and interaction effects of *Baccaurea* species and mucilaginous seed coat (MSC) on germination percentage, GP (%), mean germination time, MGT (day) and germination index, Gi

| Factor | | GP (%) | MGT (day) | Gi |
|---|---|---|---|---|
| Species | *B. polyneura* | 94.00a | 9.49b | 3.12a |
| | *B. motleyana* | 93.75a | 21.03a | 1.02b |
| MSC | Intact | 88.29b | 18.81a | 1.87a |
| | Removed | 99.43a | 13.36b | 1.98a |
| Species | | ns | ** | ** |
| MSC | | ** | ** | ns |
| Species* MSC | | ns | ** | ** |

Note: **Significant at 1% probability level, *Significant at 5% probability level, ns: Not significant. Means in each column with the different letters within each factor indicate significant differences at P≤0.05% level according to Tukey's HSD (Mean ± S.E; n=4)

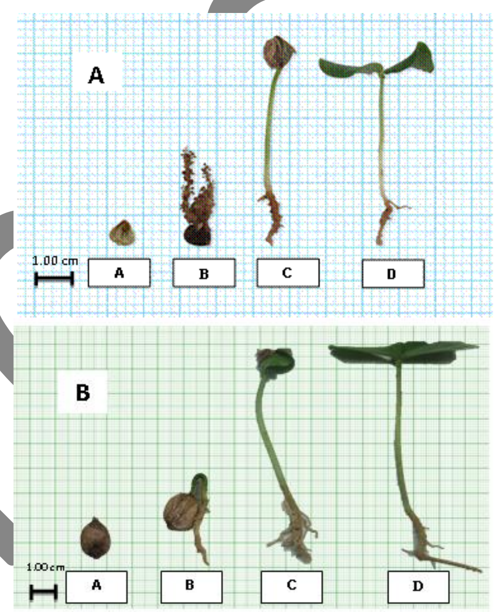


**Fig. 1:** Four categories of *B. polyneura* (A) and *B. motleyana* (B) seedlings after 30 days of germination; A (ungerminated seed), B (abnormal seedlings), C (seedlings with plumule still intact with testa), and D (seedlings with fully expanded plumule)

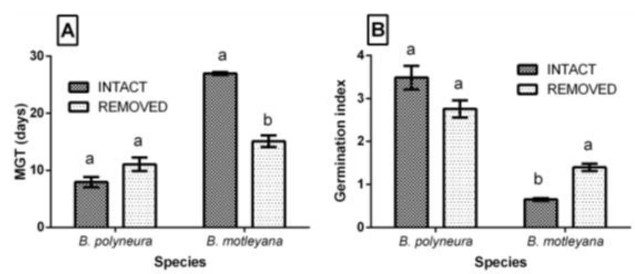


**Fig. 2:** Effects of mucilaginous seed coat on mean germination time (A) and germination index (B) of *B. polyneura* and *B. motleyana*. Means in each graph with the different letters indicate significant differences at P≤0.05% level according to Tukey's HSD. (Mean ± S.E; n=4)

**Table 2:** Main and interaction effects of *Baccaurea* species and moisture content (%) on germination percentage, GP (%), mean germination time, MGT (day) and germination index, Gi

| Factor | | GP (%) | MGT (day) | Gi |
|---|---|---|---|---|
| Species | *B. polyneura* | 76.80a | 16.41b | 1.37a |
| | *B. motleyana* | 68.50b | 21.60a | 0.75b |
| Moisture content (%) | Fresh | 98.50a | 13.51c | 1.81a |
| | 40 | 96.50a | 18.36b | 1.31b |
| | 30 | 91.38a | 19.11b | 1.21b |
| | 20 | 49.25b | 20.26b | 0.69c |
| | 10 | 25.13c | 23.79a | 0.27d |
| Species | | ** | ** | ** |
| Moisture content (%) | | ** | ** | ** |
| Species*Moisture content | ** | ** | ns | |

Note: **Significant at 1% probability level, *Significant at 5% probability level, ns: Not significant. Means in each column with the different letters within each factor indicate significant differences at P≤0.05% level according to Tukey's HSD (Mean ± S.E; n=4)

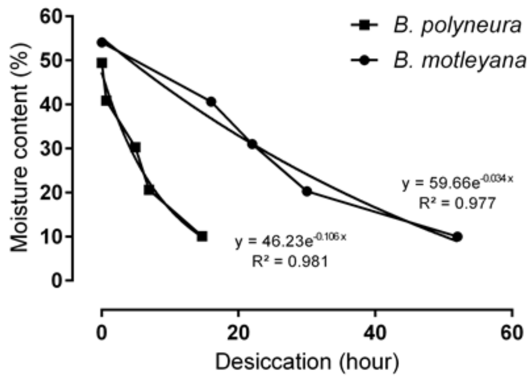


**Fig. 3:** Desiccation period (hour) for *B. polyneura* and *B. motleyana* seeds to achieve targeted moisture content (%)

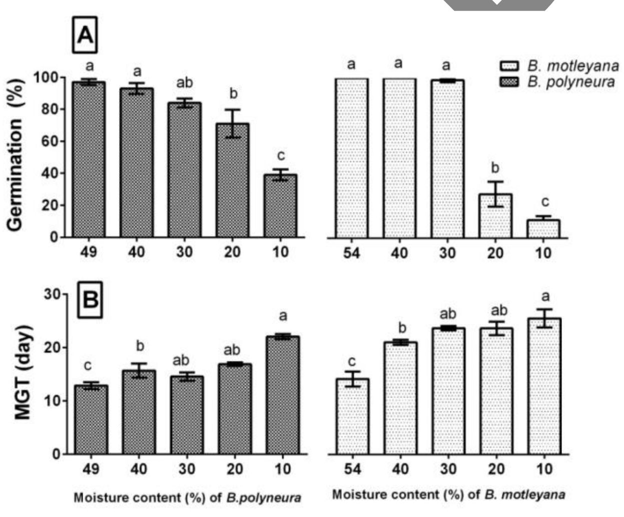


**Fig. 4:** Effects of desiccation on germination percentage (A) and mean germination time (B) of *B. polyneura* and *B. motleyana*. Means in each graph with the different letters indicate significant differences at P≤0.05% level according to Tukey's HSD. (Mean ± S.E; n=4)

Desiccation to 20% MC resulted in significant reduction of Gi by 61.8%, compared to Gi at initial MC. When further desiccated to 10% MC, the Gi in both species reduced significantly by 85.1% as compared to Gi at initial MC (Table 2). Significant interactions for germination percentage revealed that effects of desiccation on germination percentage were highly dependable on the species. Desiccating the seeds from 30% to 20% MC did not significantly reduce the germination percentage in *B. polyneura.* In *B. motleyana* however, desiccation from 30% to 20% MC significantly reduced germination percentage by 72.15%, compared to germination percentage at 30% MC (Fig. 4A).

In this study, the same pattern of mean difference classification for MGT can be observed in both species as desiccation progressed. However, degree of MGT that showed drastic increased was at difference desiccation interval in both species. Drastic increase (30.7%) in MGT for *B. polyneura* seeds can be observed at the further end of desiccation interval; when the seeds were desiccated from 20% to 10% MC. On the other hand, in *B. motleyana*, drastic increased (48.75%) in MGT was recorded at early desiccation interval; when the seeds were desiccated from initial MC to 40% MC (Fig. 4B). This showed that in view of MGT, *B. motleyana* seeds were more sensitive to desiccation. This can be supported by its higher value of correlation coefficient ($r = -0.87$, $p \leq 0.01$) between MC and MGT (Table 3) as compared to correlation coefficient value in *B. polyneura* ($r = 0.82$, $p \leq 0.01$) (Table 4).

In terms of desiccation, it seems to affect seed viability of both *Baccaurea* species, but to greater extent in *B. motleyana.* Our findings showed that both species had reduced vigor as desiccation progressed, but the effects were more explicit in *B. motleyana* compared to *B. polyneura*, as explained by the categorization of seedlings of both species based on the level of or stage of germination attained at the end of 30 days. Results showed that both main effects had significant effects ($P<0.05$) on seedling categorization, with significant interactions recorded between them (Table 3). Significant interactions between species and MC indicated that percentage of seedling that is categorized as A, B, C, D at each desiccation levels was highly dependable on species. As desiccation progressed, percentage of seedlings categorized as C and D reduced in both species, but the reduction degree varied between the species.

At initial MC, most of the seedlings from both species were categorized as C, with *B. polyneura* having significantly higher percentage of C seedlings compared to *B. motleyana*. No significant differences between the species were recorded for the rest of seedling categories. When the seeds were desiccated to 30, 20 and 10% MC, none of the seedlings from *B. motleyana* was categorized as D and the percentage value were significantly lower as compared to *B. polyneura* seedlings categorized as D at 30, 20 and 10% MC. Results also showed that at 20 and 10% MC, percentage of ungerminated seed (A category) was significantly higher in *B. motleyana*, compared to *B. polyneura* (Fig. 5).

**Table 3:** Main and interaction effects of *Baccaurea* species and moisture content (%) on seedling categorization (%)

| Factor | | Seedling Category (%) | | | |
|---|---|---|---|---|---|
| | | A | B | C | D |
| Species | *B. polyneura* | 23.0b | 6.40b | 56.0a | 20.0a |
| | *B. motleyana* | 32.70a | 31.0a | 31.5b | 4.75b |
| Moisture content (%) | Fresh | 21.25c | 8.625b | 70.88a | 18.38a |
| | 40 | 3.00c | 34.75b | 56.75a | 5.50b |
| | 30 | 8.63c | 21.75ab | 65.13a | 4.50b |
| | 20 | 50.75b | 18.25ab | 22.50b | 8.50ab |
| | 10 | 74.87a | 10.12a | 4.00c | 11.00ab |
| Species | | ** | ** | ** | ** |
| Moisture content (%) | | ** | ** | ** | ** |
| Species*Moisture content | | ** | ** | ** | ** |

**Table 4:** Pearson's linear correlation coefficients (r) between moisture content, viability and vigour parameters and seedling's categories of *B. motleyana*

| No | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|---|
| 1 | MC | - | 0.96** | -0.87** | 0.85** | -0.85** | 0.15ns | 0.80** | 0.66** |
| 2 | Gi | | - | -0.89** | 0.88** | -0.87** | 0.099ns | 0.85** | 0.73** |
| 3 | MGT | | | - | -0.60** | -0.60** | 0.13ns | -0.61** | -0.85** |
| 4 | GP | | | | - | -0.99** | 0.45* | 0.87** | 0.35ns |
| 5 | A | | | | | - | -0.47* | -0.87** | -0.35ns |
| 6 | B | | | | | | - | 0.01ns | -0.46* |
| 7 | C | | | | | | | - | 0.48* |
| 8 | D | | | | | | | | - |

MC: moisture content; Gi: germination index; MGT: mean germination time; GP: germination percentage; A: category A: seedlings; B: category B seedlings; C: category C seedlings; D: category D seedlings
**Significant at P<0.01, *Significant at P<0.05, ns: not significant

This showed that the decrease in normal seedlings was associated with a corresponding increase in ungerminated seeds, but the relationship differs greatly between species. Correlation analysis between desiccation levels and the seedling categories which was conducted based on species showed significant positive correlation ($r = 0.66$, $p \leq 0.01$) between MC and seedling category D (%) in *B. motleyana* (Table 4), whereas no significant correlation was recorded in *B. polyneura* (Table 5). This showed that as desiccation progressed, seed deterioration was more profound in *B. motleyana* as compared to *B. polyneura*.

## Discussion

Regardless of the species, removing MSC significantly increased the germination percentage by 12.62%. The presence of mucilage envelope is often associated with higher germination percentage. This effect however, can be also species-specific and influenced by several physical factors that affect the germination rate (Bhatt *et al.*, 2016). Mucilagenous seed coat can act as a physical barrier for access of water as well as oxygen diffusion, and consequently preventing germination which took place in unfavorable conditions (Yang *et al.*, 2012). Removal of mucilage layer facilitated water imbibition through direct contact, with an increase in metabolic activities within the seed. Thapliyal *et al.* (2008) reported that germination of seeds of *Dillenia indica* (Dilleniaceae) was improved when MSC was washed off from the seeds, thus allowing the seeds to imbibe water and germinate. Presence of MSC was also reported to lead physiological deterioration with time. This deterioration might be result of possibly impaired mechanisms such as structure of enzyme alterations, degradation of cell membranes, and release of phenolic compounds which leads to the reduce of enzyme activity (Chin *et al.*, 1984). MSC is also reported to be associated with fermentation which resulted in reduced germinability and vigour in the seeds covered with mucilage. Cocoa mucilage for instance consists of about 87% water, 15% sugar, 3% pentosans, 3% citric acid, and 1.5% pectin. Proteins, amino acids, vitamins (mainly vitamin C), and minerals are also present, making the seed a rich medium for microbial growth (Adu *et al.*, 2017). Although the seeds within the pod are microbiologically sterile, the mucilage will be contaminated once the pod is opened and subsequently contribute to fermentation. Fermentation will produce ethanol and acetic acid which can penetrate into the seed and eventually kill the embryo (Doyle *et al.*, 2007). In present study, removing MSC seems to be crucial in enhancing seed vigor of *B. motleyana* rather than in *B. polyneura*. There might be difference in substances of MSC of these two species which consequently differentiate the effects of removing the MSC layer. Generally, mucilage layer is produced by mucilage secreting cells which are parts of the seed or fruit coat (Kreitschitz and Gorb, 2018). This gel-like envelope around the seed is formed as a result of hydration. The increase of seed mass after hydration and mucilage formation can range between species (Sun *et al.*, 2012).

**Table 5:** Pearson's linear correlation coefficients (r) between moisture content, viability and vigour parameters and seedling's categories of *B. polyneura*

| No | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|---|
| 1 | MC | - | 0.89** | -0.82** | 0.87** | -0.87** | -0.17ns | 0.85** | -0.37ns |
| 2 | Gi | | - | -0.96** | 0.94** | -0.94** | -0.25ns | 0.91** | -0.33ns |
| 3 | MGT | | | - | -0.89** | 0.89** | 0.33ns | -0.88** | 0.32ns |
| 4 | GP | | | | - | -0.99** | -0.31ns | 0.96** | -0.23ns |
| 5 | A | | | | | - | 0.31ns | -0.96** | 0.30ns |
| 6 | B | | | | | | - | -0.36ns | -0.37ns |
| 7 | C | | | | | | | - | -0.47* |
| 8 | D | | | | | | | | - |

MC: moisture content; Gi: germination index; MGT: mean germination time; GP: germination percentage; A: category A seedlings; B: category B seedlings; C: category C seedlings; D: category D seedlings

**Significant at P<0.01, *Significant at P<0.05, ns: not significant

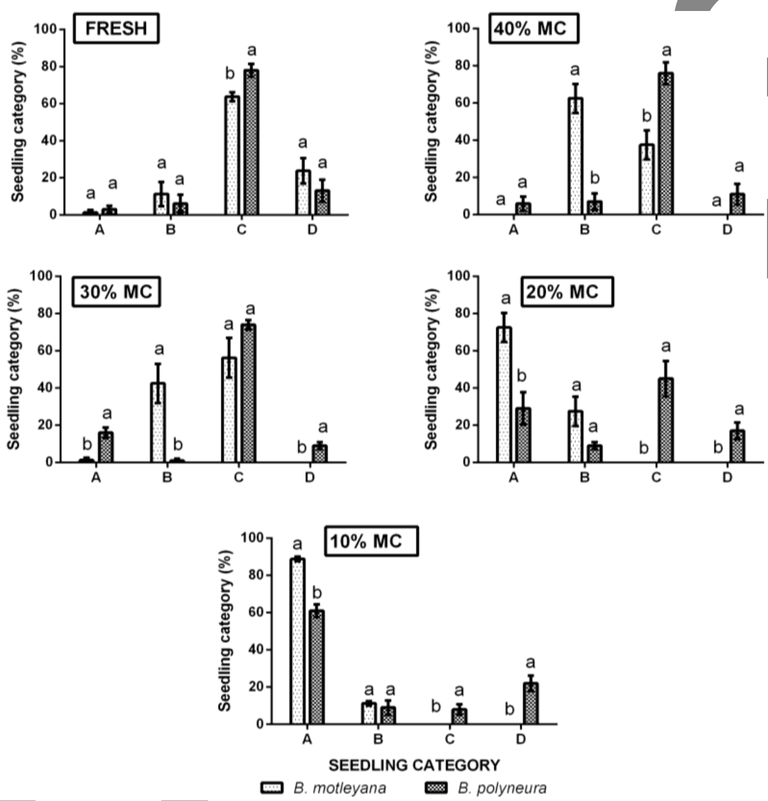


**Fig. 5:** Effects of desiccation on percentage of seedling scored into different categories for *B. polyneura* and *B. motleyana*. Means in each graph with the different letters indicate significant differences at P≤0.05% level according to Tukey's HSD. (Mean ± S.E; n=4)

Presumably, mucilagenous layer in *B. motleyana* is thicker compared to *B. polyneura*, thus affecting seed vigour of *B. motleyana* seeds.

Desiccation rate was often associated with the size attributes of the seeds. The probability of desiccation sensitivity increases as seed mass increases while it decreases with increase in seed coat ratio (Daws *et al.*, 2006). This is in agreement with findings in this study. Camellia *et al.* (2015) reported that *B. polyneura* seed attributes; length, width and thickness were significantly smaller compared to *B. motleyana* by 37.7, 31.4 and 39.7%, respectively. This justifies the faster desiccation rate of *B. polyneura* seeds as compared to *B. motleyana*.

In addition, present study results are supported by Normah *et al.* (1997), where *B. polyneura* desiccated faster compared to *B. motleyana*. In their study, desiccation for 48 h in *B. polyneura* reduced 68.17% of its initial MC whereas in *B. motleyana*, desiccation for 56 h reduced only 49.2% of its initial MC.

The pattern of desiccation rate in each quartile, the rate of desiccation for both species was slightly higher during the first quarter of desiccation period which declined at a rate of 12.76% and 1.61% MC per h for *B. polyneura* dan *B. motleyana*, respectively. However, for desiccation beyond 7 h in *B. polyneura* and 30 h in *B. motleyana*, the desiccation rate lessened to only 1.35% and 0.46% MC per h, respectively. This pattern of MC loss can be associated to the water chemistry in the seed (Alpert and Oliver, 2002). The rapid loss in MC is because of loss in free water whereas the slower rate is associated to lose in either loosely or tightly bound water (Asomaning and Sacande, 2019). Based on the decline pattern of MC (Fig. 3), further reduction of MC in *B. motleyana* seed will be difficult as the MC will hardly decline whereas in *B. polyneura* seeds, further desiccation would still be possible.

Reduction in all parameters recorded as desiccation progressed can be explained by the properties of water in seed tissue during desiccation. There are five types of water hydration levels in seed tissues as described by Vertucci (1990). At each different hydration levels, different metabolic processes can take place. At the high water contents, seeds can germinate due to full normal metabolism processes that occur. As the water content becomes lower, synthesis of protein and nucleic acid as well as respiration are possible, but there is insufficient water for cell growth and germination (Pammenter and Berjak, 2000). At further lower water contents, some respiration can be detected but synthesis of protein and nucleic acid are not possible. At even lower water contents only low level catabolic events occur slowly (Vertucci, 1990).

The results of *B. motleyana* seeds being sensitive to desiccation can be associated with the desiccation rate of *B. motleyana*, which is slower compared to *B. polyneura*. Drying rate of whole seeds had significant influence on the response to desiccation, whereby seeds that being dried rapidly withstanding desiccation to low MC (Pammenter *et al.*, 1998). Slow drying leads to homogeneous dehydration, resulting to membrane degradation. Furthermore, since the degradative processes are aqueous-based and oxidative in nature, membranes are susceptible to damage during slow drying (Pammenter *et al.*, 1998). However, if seed is dried rapidly, only a short period of time is spent at these intermediate water contents, so damage accumulation is little. This means that the faster the drying, the less damage that accumulates and to withstand with the lower water content.

Deterioration in seed is a progressive and relentless process, acting on the seed metabolism through biochemical events, leading to membrane degradation and decreased biosynthetic reactions, which result in losses of several performance qualities; germination rate, field emergency, escalation of abnormal seedlings and, eventually, loss of the germination capacity (Jyoti and Malik, 2013). Deterioration in desiccated King Palm (*Archontophoenis alexandrae*) seeds resulted in abnormal seedling percentage, largely in the intermediary moisture levels (31.5% to 28.2%) and is associated with superficial lesions which caused reduction in this seedling type and an increase in dead (Martins *et al.*, 2003). Several studies (Pritchard, 1991; Berjak and Pammenter, 1997) reported that seeds dried at intermediate moisture could suffer deleterious aqueous-based reactions, leading to poor germination rate and increased in abnormal seedlings.

In determining seed storage behavior, it is important to know whether the species shows orthodox, intermediate or recalcitrant seed storage behavior. Hong and Ellis (1996) had developed protocols in determining seed storage behavior which covers seed desiccation tolerance to low MC and its survival during storage in different environments (Hong *et al.*, 1996). With limited supply of seeds, only managed to follow the first step of the protocol; seed desiccation tolerance. Nonetheless, it would serve as preliminary information on seed storage behavior of *B. motleyana* and *B. polyneura*. Moreover, using ambient relative humidity and temperature to dry seed to about 12-18% MC such as in present study has been commonly practiced and is sufficient in differentiating recalcitrant seed storage behavior from orthodox and intermediate seed storage behavior (Hong and Ellis, 1996).

Roberts (1973) described recalcitrant seeds those cannot survive desiccation below comparatively high (between 12 and 31%) MC. In this study, desiccating the seeds from 30% to 20% MC did not significantly reduce the germination percentage in *B. polyneura*. In *B. motleyana* however, desiccation from 30% to 20% MC significantly reduced germination percentage from 98.75% to 27.5%, respectively. Further desiccation to 10% MC significantly reduced the germination percentage to only 11.25%. Moreover, none of the seedlings germinated at 20% and 10% MC were categorized as C and D (normal seedlings).

With this attributes, *B.motleyana* can be likely classified as recalcitrant while *B. polyneura* can be likely classified between intermediate and recalcitrant, which should be further confirmed by studying their responses towards different storage environments. It is not uncommon for two species which fall under the same genus to have different seed storage behavior since previous studies had reported the same. Seeds of *Coffea* species have a continuum of seed storage behaviour that ranges from recalcitrant to intermediate and then to orthodox (Eira *et al.*, 2006). *Acer saccharum* and *A. opalus* (Gleiser *et al.*, 2004) produce orthodox seeds, while *A. saccharinum*) produce recalcitrant seeds (Greggains *et al.*, 2000).

## Conclusion

*B. polyneura* and *B. motleyana* seeds differ markedly in response to removal of MSC, desiccation rate and tolerance. Removing MSC can significantly enhance seed viability of both species, but vigor was more profound in *B. motleyana*. Seeds of *B. motleyana* took longer time to be desiccated and are more sensitive to desiccation as compared to *B. polyneura*, indicated by deterioration of its viability and vigor as desiccation progressed. In terms of seed storage behavior, *B. motleyana* can be likely classified as recalcitrant while *B. polyneura* can be likely classified as intermediate, in regards of desiccation tolerance. Further study on seed storage sensitivity towards different storage environment conditions can be done to obtain comprehensive information on storage behavior of these two species.

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